STUDIES ON THE PHARMACOLOGY AND TOXICOLOGY OF THE CHLORINATED HYDROCARBON, CHLORDAN

BY

HAROLD TORREY WIEBE

A.B., Greenville College, 1939
A.M., University of Illinois, 1940

AN ABSTRACT OF A THESIS

SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
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R. UNIV. BIBLIOTHEEK LEIDEN

STUDIES ON THE PHARMACOLOGY AND TOXICOLOGY OF THE CHLORINATED HYDROCARBON, CHLORDAN

The purpose of the present investigation has been to explore the pathology and mode of action of chlordan, a chlorinated hydrocarbon insecticide, upon vertebrate and invertebrate animals. Studies were conducted on toxicity, neuro-muscular physiology, histopathology, and blood sugar levels.

Materials and Methods

Two animal types were used in this study, — the white rat, *Rattus albus,* a warm-blooded vertebrate representative, and crayfish, principally *Cambarus virilis,* as the cold-blooded invertebrate representative. The use of these two types has made possible a comparative study in several areas.

Chlordan emulsions for crayfish intravascular injections were made with (1) an emulsifier, Tween 60, and (2) crayfish Ringer. Chlordan solutions for intragastral injections of rats were made with corn oil.

For the investigation of neuro-muscular effects, kymographic recordings were made of pincer contraction in the intact crayfish and in the isolated pincer. In the latter case perfusion fluids were used. Both spontaneous and electrically elicited responses were studied.

Histological technics included fixation with AFA for general morphological staining using (1) Harris' hematoxylin and eosin, and (2) Heidenhein's iron hematoxylin and eosin; fixation with formol-calcium-cadmium for fat staining with (1) Oil Red O and (2) Sudan IV; and fixation with Carnoy's solution for glycogen staining using (1) Best's Carmine, (2) Iodine, (3) Periodic Acid Leuco-fuchsin, and (4) a Modified Best's Carmine technic. Both saliva and malt diastase were used in glycogen digestion tests. Da Fano's Cobalt

Silver Method was used to study cell inclusions.

Sections were made from frozen tissues (Adamstone and Taylor, 1948), gelatin embedded material for frozen sections (Baker, 1944-'45), and paraffin embedded materials.

Nuclear material was tested in disintegrating pigmented cells by a modification of the Feulgen reaction for frozen sections.

The "Rapid Photocolorimetric Micro Procedure for Blood Sugar Using Copper Reduction with Perchloric Acid Deprotenized Filtrates" (Polis and Sortwell, 1946) and "An Improved Form of Folin's Micro Method for Blood Sugar Determinations" (Folin and Malmros, 1929) modified by D'Amour and Blood (1948) were used in making the blood sugar determinations.

Results

1. Approximately 50 per cent of *Cambarus virilis* were killed by the fifth day through intravascular injections of chlordan (10 mg. chlordan per kg. of body weight). Males usually succumbed in about 2.7 days and females in about 3.5 days after injection.

2. Chlordan intoxication among crayfish resulted in hyperexcitability, incoordination, reduction of respiratory movements, active closing and opening of pincer, convulsive seizures, and generalized tetanus. Death which included the loss of muscle tone was preceded by a gradually declining spasticity.

3. Some intoxicated crayfish showed alternate symptoms of intoxication and recovery.

4. Crayfish subjected to an external environment containing chlordan showed symptoms of intoxication.

5. Chlordan perfusion of an isolated pincer caused (a) spontaneous sustained contraction, (b) a rise in tonus induced by a concentration of 1.0 mg. chlordan per cc.; 10 mg. per cc.

in the perfusate induced a rapid rise in tonus, and (c) a lowering of the threshold in response to electrical stimulation.

6. Electrical stimulation of a chlordan-perfused nerve showed a brief increase in excitability followed abruptly by a rise in the threshold level.

7. A rise in muscle tone was induced in an isolated pincer independent of electrical stimulation even after perfusion with atropine for anaesthetization.

8. Chlordan toxicity caused cloudy swelling of cells, pyknosis, karyorrhexis, karyolysis, and necrosis in crayfish hepatopancreas and the rat liver and kidney.

9. Liver of a chlordan-intoxicated rat showed sinusoidal congestion with disruption of hepatic cords, dispersion of bile pigments, and degeneration of the blood vessel wall.

10. Renal histopathology of the rat showed extensive glomerular congestion, glomerular tuft shrinkage, and necrosis of tubular epithelium.

11. The histological fat picture of the hepatopancreas of non-injected animals indicated that the variation can be from positive to negative — the longer animals are starved the lower the fat content. Chlordan-injected crayfish showed a variable medium to positive picture, with a 123-day starved animal being strongly positive.

12. The liver of a chlordan-intoxicated rat showed an increase in visible fat content.

13. A modified Best Carmine technic was introduced for glycogen staining.

14. Glycogen was present in the hepatopancreas during the intermolt period of *Cambarus virilis* even after prolonged starvation.

15. No significant change was observed in the glycogen picture of the hepatopancreas of chlordan-intoxicated crayfish.

16. Chlordan-intoxicated rats showed glycogen mobilization in the liver. This mobilization was first evident in the periportal area.

17. Striated muscle of chlordan-injected crayfish showed a higher degree of stainable glycogen than in like muscle of a non-injected animal.

18. Striated muscle of a chlordan-treated rat showed relatively less glycogen than striated muscle of the normal animal.

19. Da Fano's Cobalt Silver Method showed that nuclei of the hepatopancreas took the stain but rat liver nuclei did not.

20. Blood sugar determinations of crayfish starved and kept in captivity from 2 to 69 days vary from 70.21 to 138.70 mg. per cent with a 35-day starved animal showing the former and a 32-day starved animal showing the latter value. Determinations were made in April, May, August, and September; the maximum value occurred in the latter part of April and the minimum value in the early part of May.

21. Chlordan-injected crayfish gave blood sugar readings comparable to the values of non-injected animals. This was true even for readings made at varying intervals following injection.

Conclusions

1. The median lethal dose of chlordan (approximately 10 mg. per kg.) for *Cambarus virilis* given intravascularly is less than one for rabbits or mice (40-50 mg. per kg.).

2. A period of 4 to 6 days is a practicable time-span for checking the median lethal dose of chlordan upon *Cambarus virilis*, and there is some indication that males are less tolerant of the toxicant than females.

3. It is very probable that crayfish take up chlordan from the aquatic environment containing the toxicant both by way of the gills and the chitinous exoskeleton.

4. The exact mode of action of chlordan in crayfish is not clear, but it is evident that its effect is multiple. It appears that both the central and peripheral nervous systems are affected and that there is also a direct effect upon muscle tissue, the neuro-muscular junction, or both. Flaccidity in death of an intoxicated crayfish is contrasted with rigidity in death of a chlordan-treated rat.

5. Alternate symptoms of intoxication and recovery among chlordan-treated crayfish is tentatively concluded to be due to reabsorption of chlordan earlier contained in fat droplets in an inactivated state but later liberated with digestive juice by the hepatopancreas.

6. The winter season does not necessarily imply a negative fat picture of the hepatopancreas among non-intoxicated *Cambarus virilis,* and it is tentatively concluded that chlordan intoxication has an "unmasking" effect upon the fat.

7. Chlordan intoxication apparently causes fatty infiltration in the hepatic cells of the rat. This condition is correlated with liver pathology due to a toxic substance.

8. Glycogen can be present in the hepatopancreas during the intermolt period in *Cambarus virilis* even after prolonged starvation, and there is no significant change in the glycogen picture of this organ due to chlordan intoxication.

9. Chlordan intoxication results in the mobilization of liver glycogen in the rat. This mobilization is thought to be due in large measure to supra-renal stimulation.

10. Chlordan induces tonic and clonic spasticity in the rat and appears to stimulate secretion of the supra-renals resulting in depletion of glycogen stores of striated muscle. In chlordan-treated crayfish stainable muscle glycogen is increased. Some suggested explanations for this phenomenon are given.

11. A modified Best Carmine technic for staining glycogen is presented which is both rapid and relatively simple.

12. Da Fano's Cobalt Silver Method can be successfully used as a histopathological technic and has brought to light a unique nucleo-cytoplasmic relationship.

13. Starvation of crayfish kept in captivity has no significant effect upon the blood sugar level, at least within a 69-day period. Determinations are considerably higher than values given by Hemmingsen for crayfish well fed for a time, and it is concluded that a regulatory mechanism is functioning to maintain the level at about 101 mg. glucose per 100 cc. blood.

14. Chlordan does not significantly alter the blood sugar level in crayfish, which is strikingly contrasted with hyperglycemia developed in the rat.

15. Chlordan appears to be a general cytotoxin producing its effect first on the nervous system and subsequently on the vascular system. Extensive damage occurs in the hepatopancreas, liver, and kidney; a great deal of the cellular necrosis produced in these organs is possibly secondary and results from injury produced in the vascular system.

VITA

Harold Torrey Wiebe was born August 22, 1913, at Chicago, Illinois. He attended elementary schools in Hillsboro, Kansas and in China. Both elementary and high school work done in China were under American supervision. Upon graduating from Tabor Academy, Hillsboro, Kansas in 1934 he entered Central College, McPherson, Kansas, and in 1936 transferred to Greenville College, Greenville, Illinois from which institution he was graduated *cum laude* in 1939 receiving the degree of Bachelor of Arts.

He received a scholarship with stipend to the University of Illinois and was granted by that institution the degree of Master of Arts in Zoology in June, 1940.

From 1940 to 1946 positions held were: Teacher, Tabor College, Hillsboro, Kansas; Principal, Baxter School, Pueblo, Colorado; Educational Director, Civilian Public Service, Weeping Water, Nebraska; and Associate Professor of Biology, Greenville College, Greenville, Illinois. In his last year at Greenville College he was Chairman of the Division of Mathematics and Natural Science.

From 1946 to 1948 he served as graduate assistant in the Zoology-Physiology Department at the University of Illinois and became a Fellow in Zoology in September, 1948.

He has been elected to Alpha Kappa Sigma, to the Rho Chapter of Phi Sigma, and to Sigma Xi.